

REMARKS

Claims 4, 5, 8 and 12-15 are pending in the application. Claims 4, 5, 8 and 12-15 have been rejected.

Claims 4, 5 and 8 and 12-15 have been rejected under 35 U.S.C. §112, first paragraph. The Examiner contends that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

In particular, the Examiner states:

It is the Examiner's position that, as Applicant has disclosed only one embodiment of the antibody of the claims, using only said single embodiment, Applicant cannot accurately estimate the size of the antibody genus of which said antibody is a species. Additionally, chimeric antibodies consist of more than just a collection of amino acid fragments, i.e., CDRs. Antibodies comprise complex three dimensional structures in which the CDRs must fit in precise three dimensional space to create an antibody specific for any particular ligand. It is well-known in the immunological arts that the substituting of CDRs into a random framework is highly unlikely to result in an antibody of the same specificity as that of the antibody from which the CDRs were derived. Chimeric antibodies are actually constructed by trial and error starting with a framework that appears to resemble that from which the CDRs were derived. Accordingly a written description that consists only of the CDR regions (and in the case of Claims 4, 5, 8, 14 and 15, just half of the CDR regions) is inadequate to describe the CD25 binding molecule of the instant claims.

Applicants respectfully disagree with the Examiner's conclusion and submit that the specification contains sufficient written description of a CD25 binding molecule as set forth in amended independent Claim 4.

Applicants reiterate the arguments proffered in the previous response to address this rejection.

It is further noted as set forth in the MPEP §2163 (I) that to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. MPEP §2163 (II)(A)(3)(a)(i) further states that whether the specification shows that application was in possession of the claimed invention is not a single, simple determination, but instead is a factual determination reached by considering a number of factors such as level of skill and knowledge in the art, partial structure, and method of making the claimed invention.

Applicants assert that while one specific embodiment of the CD25 binding molecule recited in the claims is disclosed in the present application as argued by the Examiner, such an embodiment coupled with the level of skill in the art, knowledge in the art and a method of making the claimed antigen binding molecule would lead one skilled in the art to recognize that applicant was in possession of the presently claimed method.

The CD25 binding molecule recited in the presently claimed method is described by CDRs having specific sequences. These CDR sequences in defining the antigen binding site

are the relevant identifying characteristics of the recited antibody. The CD25 binding molecule possessing these specific CDR sequences can take on different forms. The CD25 binding molecule can be a chimeric CD25 antibody, wherein the complete variable domains of one antibody are linked to constant domains derived from another antibody. In an embodiment described in the present specification, the complete variable domains are from a mouse Mab and the constant domains are from a human immunoglobulin. A method of producing a CD25 binding molecule having the specified CDR sequences is described in EP 449,769, which is incorporated by reference into the present specification.

The CD25 binding molecule having the specified CDRs can also be a humanized antibody. Typically, the humanized antibody refers to an antibody having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin-derived parts of the antibody being derived from a human immunoglobulin. The antigen binding site typically comprises CDR which determine the binding specificity of the antibody molecule and which are carried on appropriate framework regions in the variable domains.

As indicated by the Examiner it is well-known in the immunological arts that the substituting of CDRs into a random framework is highly unlikely to result in an antibody of the same specificity as that of the antibody from which the CDRs were derived. At the time of filing of the present application, however, a number of successful methods for making humanized antibodies were well-known in the art. These methods, rather than substituting CDRs into a random framework, involve the selection of specific amino acids for the framework region to maximize binding of the humanized antibody to the antigen. For example, the specified CDRs from a murine antibody can be grafted into the DNA coding for the framework of a human antibody as described, e.g., in EP-A-0239400 (Winter). Another example of a method for selecting specific amino acids for the framework region to improve binding affinity of the humanized antibody is described in detail, e.g., in U.S. Patent 6,180,370 (Queen). Various methods for producing humanized antibodies having improved binding affinity that were state of the art at the time of filing of the present application are also summarized, e.g., in Vaswani et al., "Humanized antibodies as potential therapeutic drugs" *Ann. Allergy Asthma Immunol.* 81: 105-119, 1998, a copy of which is attached (see pages 106-108). Accordingly, rather than the method of producing framework regions being a random, trial and error approach as stated by the Examiner, there were well-known methods in the immunological arts at the time of filing of the present application to design humanized antibodies having improved binding affinity by selection of specific amino acids for the framework regions.. Accordingly, a written description of the specific, relevant, CDR regions of the CD25 binding molecule coupled with high skill and knowledge in the art of how to design such humanized antibodies possessing framework regions that can improve antibody affinity and disclosure of how to make the CD25 antibody recited in the presently claimed method would lead one skilled in the art to the conclusion that the applicant was in possession of the claimed invention.

In addition to chimeric and humanized antibodies, techniques for making fragments of the aforementioned antibodies are well known in the art. Thus, one skilled in the art would recognize that in view of the embodiment disclosed in the present application, the high level of skill in the art and knowledge in the art on how to make the aforementioned antibodies, there are a representative number of antibodies that are sufficiently described by the specific CD25 antibody recited in independent Claim 4.

In view of the above, withdrawal of the rejection of Claims 4, 5, 8 and 12-15 under 35 U.S.C. §112, first paragraph, is respectfully requested.

Claims 4, 5 and 8 have been rejected under 35 U.S.C. §103(a) as being unpatentable over WO 89/09622 (WO'622) in view of Kovarik et al. In particular, the Examiner states:

The reference [WO'622] does teach that "The present invention provides novel compositions useful in the treatment of T-cell mediated human disorders, the compositions containing a chimeric antibody specifically capable of binding to human IL-2 receptors, such as at the epitope bound by the anti-Tac monoclonal antibody. The IL-2 chimeric antibody can have two pairs of light chain/heavy chain complexes, wherein at least one pair has chains comprising mouse variable regions joined with human constant region segments, with or without naturally-associated J and D segments" (page 3) and further teaches rheumatoid arthritis (RA) as one such disease. In other words, the reference teaches the use of a chimeric anti-IL2 receptor antibody for the treatment of RA. Kovarik et al. teaches the chimeric anti-IL2 receptor antibody basiliximab which comprises the CDRs of the instant claims. Accordingly, the combined references need comprise nothing more than the substitution of obvious equivalents for a proper obviousness type rejection. However, the Kovarik et al. reference teaches more. It also teaches that basiliximab can achieve IL2 receptor saturation and that the antibody is well tolerated, thus basiliximab could be considered to be not just an equivalent of the antibody of the '622 document, but a preferred substitution for said antibody....

Applicants respectfully disagree with the Examiner's conclusion and submit that the combination of references does not make obvious Claims 4, 5 and 8 for the reasons below.

Applicants reiterate the arguments proffered in the previous Office Action to address this rejection. In particular, WO'622, while describing anti-TAC chimeric antibodies, does not teach the antibodies recited in independent Claim 4, or that such antibodies can be utilized to treat rheumatoid arthritis.

Kovarik et al., while teaching the specific chimeric monoclonal antibody, basiliximab, and the use of this antibody for immunoprophylaxis against acute rejection in renal transplantation fail to specifically suggest that binding of basiliximab to IL-2 receptor at serum concentrations sufficient to saturate the receptor to treat transplant rejection would also be effective to treat rheumatoid arthritis.

Accordingly, there is nothing in the combination of references that specifically suggests that basiliximab can be effectively utilized to treat rheumatoid arthritis.

The Examiner alleges that the basiliximab antibody described in Kovarik et al. can be considered a preferred substitute for the anti-TAC antibody described in WO'622 because basiliximab can achieve IL2 receptor saturation and is well tolerated. Thus, it is the Examiner's position that one skilled in the art would be motivated to substitute the anti-Tac antibody with basiliximab to treat rheumatoid arthritis making the presently claimed subject matter obvious.

It would appear that the Examiner's conclusion of obviousness appears to suggest that he is applying an "obvious to try" standard which has consistently been proscribed by the Federal Court. Note the Federal Court's instruction in *The Gillette Co. v. S.C. Johnson & Johnson Inc.*, 16 U.S.P.Q.2d. 1923, 1928 (Fed. Cir. 1990). *In re Eli Lilly & Co.*, 902 F.2d 943, 945, 14 U.S.P.Q.2d. 1741, 1743 (Fed. Cir. 1990), wherein the Court noted:

As we recently explained,

[a]n "obvious-to-try" situation exists when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued. *The Gillette Co. v. S.C. Johnson & Johnson Inc.*, 16 USPQ2d 1923, 1928 (Fed. Cir. 1990). *In re Eli Lilly & Co.*, 902 F.2d 943, 945, 14 USPQ2d 1741, 1743 (Fed. Cir. 1990).

Thus, while WO'622 and Kovarik et al. might arguably pique one's interest in pursuing studies to determine the effect of basiliximab in treating rheumatoid arthritis, there is no teaching in any of the references specifically suggesting how to obtain this desired result or that such a result would be obtained if certain direction were pursued. Accordingly, there is insufficient information in the cited art of record which would lead one skilled in the art to the present invention. Consequently, to suggest that the presently claimed method is obvious is nothing more than an invitation to experiment which is not allowed.

In view of the above, withdrawal of the rejection of Claims 4, 5 and 8 under 35 U.S.C. §103(a) is respectfully requested.

A good faith effort has been made to place the present application in condition for allowance.

If the Examiner believes that a telephone conference would be of value, he is requested to call the undersigned counsel at the number listed below.

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Humanized antibodies as potential therapeutic drugs

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Background: Antibodies have been used therapeutically to treat a variety of clinical conditions. The introduction of monoclonal antibodies and more recently, engineered humanized antibodies has greatly refined and expanded the therapeutic potential of this modality of treatment.

Learning Objectives: To reinforce the reader's knowledge of the therapeutic application of antibody in the treatment for different diseases. More specifically, to enhance reader's understanding of basic methods employed in the production and clinical use of humanized antibodies.

Data Source: The MEDLINE database was used to review the humanized antibody related literature.

Conclusion: Humanized antibodies provide a novel approach for the treatment of a broad range of diseases. Expanded use will depend on improvement in their efficacy (avidity and specificity), demonstration of their safety, and reduction of their immunogenicity.

Ann Allergy Asthma Immunol 1998;81:105-119.

INTRODUCTION

Antibodies are proteins produced in response to an antigen and specifically bind with the antigen that induced their formation. The use of antibodies for therapy began in 1890 when Von Behring and Kitasato discovered antidipteria toxins.¹ Over the years, specific antisera have been used for passive immunization. For instance, hyperimmune serum from patients has been used to treat infections such as rabies and tetanus. The specificity and avidity of antibody for its specific antigen has been the basis for promoting exogenous therapeutic administration of antibody. In the past 10 years, rapid advances in genetic engineering have made it possible to enhance the specificity of the antibody combining sites.

to alter its size, structure, and shape, and thus to increase the therapeutic utility of antibodies while minimizing their immunogenicity.² We review the different methods that have been employed to "engineer" the antibody and provide an illustration of how antibody can be used to treat human allergic diseases.

HISTORICAL PERSPECTIVE

Polyclonal Antibodies

The introduction of antibodies in the treatment of immunodeficiency and infectious diseases was a significant breakthrough in medicine. The initial antibody preparations available for use, such as intravenous gammaglobulins, were polyclonal. Animal and human antisera have been used in vivo to destroy bacteria (tetanus, pneumococcus) and neutralize virus (hepatitis A and B, rabies, cytomegalovirus, and varicella zoster) in the blood of infected individuals. Possibly the most important early application was the use

of antibody to the red blood cell Rh antigen produced in healthy human males to successfully clear postpartum Rh negative mother's blood of Rh positive fetal red blood cells. Polyclonal antibody treatment is associated with several unwanted side effects. There is a risk of infection from contaminating viruses that has been addressed by implementation of viral inactivation steps. Serum sickness, anaphylaxis, and the polyclonal nature of these antibody preparations have also been considered limiting factors.^{3,4} This has motivated researchers to produce well-defined, specific, and quality controlled antibodies from single clones.

Properties of an Ideal Antibody Drug

The ideal antibody drug⁵ should possess monospecificity for its target antigen such as monoclonal antibodies that can be produced with hybridoma techniques (see below). Further, it should have no detectable crossreactivity to irrelevant soluble and cell-bound antigens and have maximal avidity and immunoreactivity for its target antigen with affinity constants from 10^8 to 10^{10} L/M. It should be sterile, apyrogenic, free of detectable contaminating proteins, DNA, virus, fungi, bacteria, mycoplasma, and endotoxins. It should be non-immunogenic in man such that it does not elicit human anti-mouse antibodies (HAMA) and anti-allotype antibodies. The free antibody drug should not activate complement via the classical or alternative pathway. It should be reproducibly produced from stable cell clones that are readily stored and adaptable to large-scale culture with serum-free media (for easy puri-

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fication with minimal contamination). Finally, it should possess the ability to elicit effector functions such as antibody-dependent complement-mediated cytotoxicity (ADCC) and antibody-dependent cellular cytotoxicity (ADCC) once bound to its target antigen.

Monoclonal Antibodies

In 1975, Kohler and Milstein introduced techniques for producing hybridomas that secrete monoclonal antibodies (mAbs) of defined specificity. The mAbs possess many "ideal" properties of an antibody drug. The procedure consists of fusing antibody-producing B cells sensitized against a particular antigen with a myeloma cell line.⁹ Following selection with single cell cloning, monospecific antibodies are produced and expanded in mice. Subsequently, this method has been applied with less success to the production of mAbs in rats and humans. The affinity and specificity of highly selected mAbs have proven to be their greatest asset as reagents in diagnostic laboratory assays and as drugs in treating human diseases. The mAbs have several advantages over polyclonal antibodies and some disadvantages as noted in Table 1. One major limitation to the *in vivo* use of murine mAbs has been the induction of human anti-mouse immunoglobulin response which causes rapid clearance and reduction in effector function efficiency. Research has focused on modifying the murine mAb to attenuate its antigenicity while maintaining its avidity and specificity.¹³

Humanized Antibodies

Immunologically, the best alternative to therapeutic murine mAb is a human mAb. Even the best researchers, however, have found it difficult to produce human mAbs for clinical use with conventional hybridoma techniques.¹⁴⁻¹⁶ The primary problem has been an absence of a good human myeloma fusion partner that does not lose chromosomes with continued growth in cell culture. Alternative cellular biology approaches to immortalize B cells have also proven unsatisfactory. These have

Table 1. Rodent Monoclonal Antibodies as Therapeutic Drugs

Advantages	Disadvantages
When compared to polyclonal, the rodent monoclonal antibodies (mAbs) of desired specificity and affinity can be mass produced reproducibly.	All mAbs are primarily of rodent origin. When administered to humans, they elicit an immune response, human anti-mouse immunoglobulin (HAMA) ⁷⁻⁹
The amount of antibody protein required for immunization is less with mAbs than with polyclonal antibodies.	Neutralization of murine mAb by HAMA can result in attenuation of its therapeutic effect and accelerated clearance from the blood.
They can be more readily standardized and quality controlled.	Production of human IgE antibodies may lead to hypersensitivity reactions.
	Short circulating half-life. Require repeated administrations ¹⁰⁻¹²
	Rodent antibodies are not as effective as human antibodies with regard to eliciting human immune effector function.

included the generation of lymphoblastoid cell lines involving infection with Epstein Barr virus (EBV), a B-lymphotropic herpes virus. Once transformed, EBV-infected B-cell lines tend to grow in clumps, release virus, and secrete low levels of antibody, primarily of the IgM isotype. They also require repetitive cloning which is labor intensive. Possibly most important, they do not resolve the problem of obtaining human B cells that are secreting antibody with the desired antigen specificity and isotype (class and subclass). Ethical issues prevent the direct immunization of humans with most biologic agents and one cannot readily harvest cells from human spleens. Excised lymph nodes have not been an ideal source of human B-cells.

An alternative approach to overcoming the immunogenicity problems associated with murine mAb has been to re-engineer the antibody to make it "human-like." In order to understand the process of humanization of an antibody, it is essential to review the fundamental antibody structure. The term "humanization of an antibody," or more specifically humanization of a murine mAb, implies substitution of part of the murine antibody with segments that make the antibody "human."

First Generation/Chimeric Antibodies

The first humanized antibodies were chimeric antibodies, in which the variable region genes of a rodent antibody were cloned into a mammalian expres-

sion vector containing the appropriate human light chain and heavy chain constant region genes¹⁷⁻¹⁹ (Fig 1a). The resulting chimeric mAb (rodent + human) should have the antigen-binding capacity (from the variable region of the rodent) and should be significantly less immunogenic than the original rodent mAb.

Selection of the desired human antibody heavy chain isotypes is often based on the desired effector functions for a specific therapeutic application (Table 2). In some situations, effector functions mediated by the Fc region of the antibody are not required or may be deleterious due to inappropriate uptake by Fc receptor-bearing cells. In other cases, the constant regions from IgG2 and IgG4 isotypes may be useful since they have lower affinity for Fcγ receptors and are thus less efficient at mediating antibody-dependent complement-mediated cytotoxicity.²⁰ Alternatively, a modified human IgG might be constructed in which the Fc receptor-binding site has been altered to a low-affinity form.²⁴ A humanized murine anti-human CD3 mAb, OKT3, for instance was modified to alter the FcγR1 (CD64) binding site and it employed a gamma 4 constant region to further lower its IgG:FcγR1 binding affinity.^{23,24,26}

Allotypic Variation

To minimize the antigenicity of humanized antibodies, it is important not only to reshape the variable region but

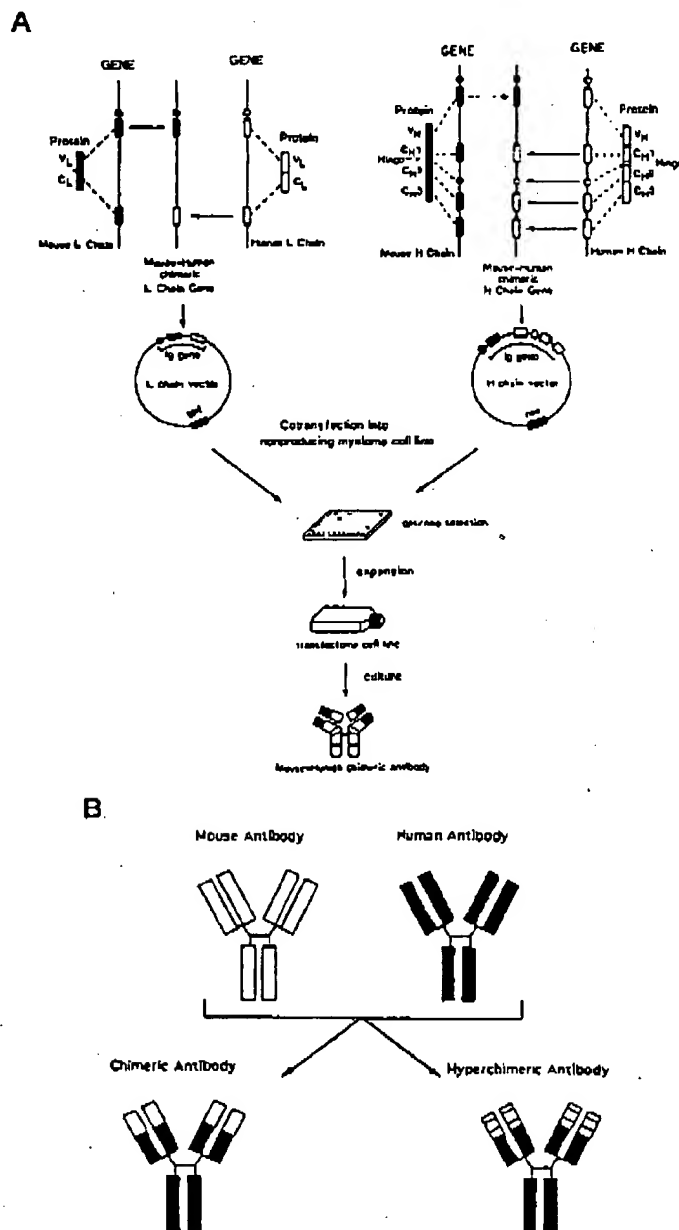


Figure 1a. Schematic presentation of chimeric antibody production. First, a human constant region and mouse variable region genes are ligated to form a chimeric gene. Subsequently, it is co-transfected into a recipient cell line, which then produces both heavy and light chains of a chimeric antibody.⁵

Figure 1b. Evolution from mouse antibody to humanized antibody (Mouse → Chimeric → Humanized). The chimeric antibody has human heavy and light chain constant regions and mouse heavy and light chain variable regions. The hyperchimeric antibody is human except for the CDRs of the mouse antibody. Reproduced with permission from Hamilton RG, Sun L, Beall PT. Production and quality control of monoclonal antibody reagents and drugs. In: Moulds JM, Musouredes SP, eds. Monoclonal antibodies. Arlington, VA: American Association of Blood Banks, 1989:100.

also to minimize the allotypic variation in the constant region. Engineering of a therapeutic antibody with different allotypes to match each patient's allotype would be impractical. One suggested alternative is to produce the humanized antibody with the most frequent allotype in the general population and accept that some patients might develop antibodies to minor allotypic differences,²² which would facilitate their clearance.

Second Generation/Hyperchimeric Antibodies

In 1986, Jones and his colleagues further humanized rodent antibodies through a technique known as complementarity determining region (CDR) grafting.²⁷ In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting rodent mAb and grafted into the DNA coding for the framework of the human antibody (Fig 1b). Since only the antigen-binding site CDRs, rather than the entire variable domain of the rodent antibody are transplanted, the resulting humanized antibody (second generation or hyperchimeric antibody) is less immunogenic than the first generation chimeric antibody.

This process has been further improved by molecular biologists to include changes referred to as "reshaping,"²⁸⁻³⁰ "hyperchimerization,"³¹⁻³³ and "veneering."³⁴ In the reshaping process on the basis of homology, the rodent variable region is compared with the consensus sequence of the protein sequence subgroup to which it belongs. Similarly, the selected human constant region accepting framework is compared with its family consensus sequence.³⁵ The sequence analyses identify residues, which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic. Inclusion of the more common human residues minimize immunogenicity problems by replacing human acceptor idiosyncratic residues. Further, the reshaping process allows comparison of human and

Table 2. Human Antibody Isotypes

Isotype	Functional Characteristic
IgG1	<ul style="list-style-type: none"> Effective for ADCC* and ADCMC† Isotype of choice for antibody production which maximizes both ADCMC and ADCC effector functions²⁰
IgG2	<ul style="list-style-type: none"> Least effective in ADCMC²¹ Ineffective for ADCC²² In certain situations, where the effector functions mediated by the Fc region of the antibody are not required, the constant regions from IgG2 (and IgG4) antibodies would be useful since they have low affinity for Fc receptors and are inefficient at mediating ADCMC²³
IgG3	<ul style="list-style-type: none"> Effective in ADCMC Also effective in ADCC (less than IgG1)
IgG4	<ul style="list-style-type: none"> More suitable for laboratory diagnostic imaging and receptor blockade function in a monovalent manner.

* ADCC = Antibody dependent cellular cytotoxicity.

† ADCMC = Antibody-dependent complement-mediated cytotoxicity.

rodent consensus sequences to identify any systematic "species" differences. RSV19 antibodies for instance have been successfully humanized by employing this procedure.^{36,37}

Hyperchimerization is an alternative method of identifying residues outside of the CDR regions that are likely to be involved in the reconstitution of binding activity. In this method, the human sequences are compared with murine variable region sequences and the one with highest homology is selected as the acceptor framework. As in the reshaping procedure, the "idiosyncratic" residues are replaced by the more commonly occurring human residues. The non-CDR residues that may be interacting with the CDR sequences are identified. Finally, we determine which one of these residues is to be included in the variable region framework. Humanized antibodies against CD33 antigen were developed by this method.³⁸ The relative affinity of the antibodies humanized by this procedure can be significantly increased when compared with murine antibodies.³⁹

The displayed surface of the protein is the primary determinant of its immunogenicity. A humanized murine antibody can thus be made less immunogenic by replacing the exposed residues, which differ from those commonly found in human antibodies. This method of humanization is referred to as "veneering."³⁴ Appropriate

replacement of the outer residues may have little or no impact on the inner domains or interdomain framework. Veneering is a 2-step process. In the first step, the most homologous human variable regions are selected and compared by each single residue to the corresponding mouse variable regions. In the second step, the mouse framework residues, which differ from its human homologue, are replaced by the residues present in the human homologue. This replacement involves only those residues that are on the surface and at least partially exposed.⁴⁰

THERAPEUTIC APPLICATION OF ANTIBODIES TO HUMAN ALLERGIC DISEASES

Limitations of Conventional Therapies

There are number of limitations to current methods of treating allergic diseases. Avoidance through environmental control measures may not be feasible due to poor compliance and they are often difficult to implement. Antihistamines are the most frequently used drugs in the pharmacotherapy of allergic diseases. In addition to histamine, numerous other inflammatory mediators are released from degranulating mast cells and basophils for which no specific antagonist is available at this time. Several leukotriene inhibitors have been reported to be ef-

fective in clinical trials. Leukotriene receptor antagonists (such as zafirlukast and montelukast) 5-lipoxygenase inhibitor (zileuton) were recently approved by the FDA for commercial use. Corticosteroids and mast cell inhibitors (cromolyn sodium and nedocromil) are also used in the treatment of allergic diseases.

The production of multiple, severe adverse effects by prolonged administration of oral corticosteroids limits their widespread and indiscriminate use. Traditional allergen immunotherapy requires numerous repeated injections of allergen over a period of years, is associated with some risk of anaphylaxis, and only few allergenic extracts are standardized biologically.

Antibody to IgE

Since IgE plays the pivotal role in type I immediate hypersensitivity allergic reactions, it is a reasonable target for immunologic modulation. Studies have shown that the level of total serum IgE tends to correlate with severity of disease, especially in asthma.^{41,42} Furthermore, the level of allergen-specific serum IgE can be used as a relative measure of the degree of the allergic sensitization to a specific allergen. In view of all this, reduction of the IgE level in the blood or blockade of IgE binding to mast cells and basophils could theoretically be beneficial in the treatment of allergic diseases without having any impact on normal immune functions.

Humanized Specific IgE Antibodies

Several investigators have tried different approaches to modify the level of circulating IgE or the quantity of IgE bound to receptors. Presta and his colleagues⁴³ have humanized a murine antibody, MaEl1, directed against IgE that prevents the binding of free IgE to FcεRI on mast cells, but that does not bind to FcεRI-bound IgE. The latter feature is extremely important for safety since this antibody will not trigger degranulation of mast cells through crosslinking of cell surface IgE on mast cells or basophils. The humanized version of this antibody, rhu Mab-

Table 3. Reported Non-Allergy Applications of Monoclonal Antibody Drugs

Disease	Humanized Antibody	Antigen	Comments	Ref.
Coronary artery disease	c7E3 Fab	Platelet glycoprotein IIb/IIIa receptor	Multi-center randomized double-blind trial of 2099 patients. Ischemic complications of coronary angioplasty reduced; risk of bleeding increased.	59
Mycosis fungoides	Anti-CD4	CD4	Anti-CD4 chimeric mAb of limited clinical efficacy in a small number of patients.	60
Non-Hodgkin's B cell lymphoma	CAMPATH-1H	CAMPATH-1; present on all lymphoid cells & monocytes but not on other cell types	Successful clearance of malignant cells from blood and bone marrow in two patients. Both relapsed. There was no antiglobulin response, and no undue side effects. Tumor cell depletion was achieved with mAb alone without other subsidiary chemotherapy or radiotherapy.	61
Systemic vasculitis	CAMPATH-1H	CAMPATH-1	Short term remissions with several courses of CAMPATH-1H. A course of CAMPATH-1H followed by a rat IgG2b-CD4 antibody resulted in long term improvement. An auto-antibody to a neutrophil antigen was no longer detectable	62
Refractory rheumatoid arthritis	CAMPATH-1H	CAMPATH-1	Clinical improvement in 7 out of 8 patients lasting 12 wk to 8 mo. with no correlation between clinical relapse and lymphocyte count. No measurable antiglobulin response, but 3 out of 4 patients were sensitized on retreatment.	63
Generalized pustular psoriasis	Anti-CD4	CD4	Clinical and histologic improvement seen in only one patient.	64
Severe psoriasis	Anti-CD4	CD4	Clinical and histologic improvement in 3 patients.	65
Cardiac transplant	Anti-Tac-H	IL-2 receptor	Cardiac allograft survival prolonged in monkeys. Potential for human use postulated.	66
Metastatic colorectal adenocarcinoma	IgG1 antibody 17-1A	Glycoprotein antigen	No tumor regression in 10 patients studied; 1 developed an antiglobulin response.	67
Rheumatoid arthritis	CD4 (CM-T412)	CD4	Open-label, non-randomized study in 29 patients. No correlation between clinical improvement and laboratory parameters. Majority of patients developed antiglobulin response to antibody.	68
Septic shock	HA-1A	Lipid A region of the gram-negative bacterial lipopolysaccharide	Randomized, double-blind placebo-controlled trial of 821 patients on HA-1A or placebo. No difference between the treatment & placebo groups at 14 days all-cause mortality.	69

E25, is currently in phase III clinical trials in children (6 to 12 year olds) and it seems to have promising clinical efficacy without noticeable deleterious side effects (Dr. Paula Jardim, personal communication by Dr. Susan MacDonald). Two mouse anti-human FcεRI monoclonal antibodies, CRA2 and CRA4, inhibit IgE binding to FcεRI. The humanized CRA2 has retained the same characteristics as its murine counterpart whereas CRA4 lost its bioactivity following humanization of heavy chain variable region. Activated basophils do not degranulate when pretreated with humanized CRA2.⁴⁴ Davis et al⁴⁴ recently reported

the development of a chimeric monoclonal antibody, CGP 51901, specific for a unique epitope on human IgE. In vitro tests showed that CGP 51901 bound only to human IgE with no binding to other human immunoglobulin isotypes. This antibody also binds specifically to IgE secreting B cells and consequently has the potential to reduce total circulating IgE levels. In addition, it does not bind to and crosslink cell surface IgE on basophils and mast cells. Hence, it maximizes safety by not inducing the release of histamine and other vasoactive mediators from IgE-bearing mast cells and basophils. CGP51901 antibody there-

fore also has the potential for therapeutic use in humans.

Additionally Chang, Davis and their colleagues⁴⁶ have prepared and screened several thousand hybridomas to find those producing high affinity anti-IgE mAbs. In the process, they discovered antigenic epitopes on IgE that are accessible on IgE-secreting B cells but not on other cells bearing IgE that are bound to the Fcε high affinity receptor. Among 42 murine mAbs specific for human IgE, TES-1, and TES-3 were shown to bind to IgE-secreting cell lines, but not to IgE bound to high-affinity FcεRI on basophils or to the low-affinity FcεRII on other cell types.

Hence, these mAbs were unable to stimulate histamine release from IgE-bearing basophils under laboratory conditions. This work has been expanded to humanize anti-human IgE antibodies. A mouse anti-human IgE mAb was humanized by CDR grafting.⁴⁷

Other potential targets toward which humanized antibodies could be directed to treat human allergic diseases include cells (mast cells, basophils, eosinophils, or possibly even Th2 type T cells), adhesion molecules (V-CAM/VLA-4), and inflammatory mediators (eg, leukotrienes).

In recent years, eosinophilic inflammation and various ways to impede the migration of the eosinophils into the airways has been the subject of intense investigation. Eosinophils bind to the vascular adhesion molecule-1 (VCAM-1). This binding is mediated by very late antigen-4 (VLA-4), the $\alpha 4 \beta 1$ heterodimeric integrin (CD49d/CD29).⁴⁸ Very late antigen-4 also mediates the binding of these cells to the CS-1 region of fibronectin, and potentially also to an additional endothelial cell ligand.^{49,50} HP 1/2 is an IgG1 murine mAb directed against the $\alpha 4$ chain of the human $\alpha 4 \beta 1$ heterodimeric integrin, VLA-4, which when used in an allergic sheep model, was shown to modify antigen-induced late bronchial response and to prolong airway hyperresponsiveness.⁵¹ The intercellular adhesion molecule-1 (ICAM-1) is also important for eosinophil adhesion to endothelium, and has been shown to be unregulated on endothelium and skin epithelium after an inflammatory stimulus.^{52,53} In a primate model of asthma, a monoclonal antibody to ICAM-1 was shown to attenuate airway eosinophilia and as well as bronchial hyperresponsiveness.⁵⁴

Along another line, interleukin-5 (IL-5) has been found to be essential for the development of blood and tissue eosinophilia.⁵⁵ An anti-IL-5 monoclonal antibody, TRFK-5, when given to BALB/c mice inoculated with parasitic larvae, completely suppressed the blood eosinophilia and the infiltration of eosinophils into the lungs of mice.⁵⁶ In another recent study, when mice

were given anti-LFA-1 and anti-ICAM-1 mAbs, increased skin graft survival occurred.⁵⁷ In addition, this study showed that in vivo injection of anti-IL-10 mAbs abolished delayed graft rejection. The therapeutic role of anti-IL-4 mAb was also suggested by results in a murine model of chronic paracoccidiosis *brasiliensis* infection.⁵⁸ Resistance to the infection correlated with decreased serum IgE. Although these latter studies did not employ humanized antibodies, once humanized antibodies with these specificities are optimized, they may be attractive targets for therapy.

OTHER DISEASES

A number of humanized antibodies with other antigen specificities have been used in clinical trials. A recently reported, prospective, randomized double-blind trial using humanized mAb c7E3 Fab against the platelet glycoprotein IIb/IIIa receptor is the largest such study undertaken to date.⁵⁹ Two thousand ninety-nine patients at high risk due to unstable angina, evolving acute myocardial infarction who were undergoing coronary angioplasty received an injection of c7E3 Fab. When compared with placebo, the ischemic complications of coronary angioplasty and atherectomy were reduced in the antibody-treated group. Therapy with mAb c7E3 Fab reduced all types of myocardial infarctions as well as the need for emergency coronary revascularization, but increased rates of major bleeding and requirements for blood transfusion were found in the antibody group. While this study is important in demonstrating the therapeutic usefulness of humanized mAb in reducing the risk of myocardial infarctions, the potential for adverse complications still exists.

The following four are the other examples of the use of humanized antibodies as therapeutic agents. (1) Anti-CD4 chimeric antibodies for treatment of mycosis fungoides.⁶⁰ (2) CAMPATH-1H, a humanized antibody against mature human leukocytes for non-Hodgkin's lymphoma. In two patients, lymphoma cells were cleared from the blood and bone marrow and

splenomegaly resolved. Both patients, however, eventually relapsed with their original lymphomas. No antiglobulin response was detected in either patient.⁶¹ (3) CAMPATH-1H for intractable systemic vasculitis.⁶² A course of CAMPATH-1H followed by a rat IgG2b CD4 antibody resulted in long-term improvement in one patient. (4) CAMPATH-1H for refractory rheumatoid arthritis. Significant clinical benefit was found in seven out of eight patients.⁶³ Additional humanized antibodies, used in the treatment of a variety of disorders, are described in Table 3. Most of the studies have shown positive therapeutic results; however, due to small numbers of patients and complications, it is premature to draw any definitive conclusions about the clinical efficacy of humanized antibodies.

Antibody Fragments

With the improved molecular biology techniques, it is now possible to genetically engineer antibody fragments. Due to their smaller size, the antibody fragments have greater penetration into tumor tissues and are more rapidly cleared from the non-tumor tissue.⁷⁰ Antibody fragments can be produced economically using a bacterial expression system. Specific, high affinity human antibody fragments can be developed de novo from naive immunoglobulin gene libraries.⁷¹ When compared with intact antibody, the smaller antibody fragments [F(ab')₂, Fab] bind to tumor tissue more homogeneously. With antibody fragments, a higher time-dependent tumor: normal tissue ratio of antibody can be achieved with a lower dose.⁷²

Radioisotope, Drug and Toxin Conjugated Antibodies

Antibody fragments have been linked to radioisotopes and toxins, primarily for use in the cancer therapy. Radioisotopes can be bound to the antibody randomly or at the hinge region by specific chemical linkage. One advantage of radiolabeled antibody conjugate is that it can kill malignant tumor of several cell diameters, thereby over-

Table 4. Recent Reports on Antibody Drug Therapy

Disease	Humanized Antibody	Antigen	Comments	Ref.
Rheumatoid arthritis	cA2	TNF- α	Single infusion of cA2 resulted in elevation of CD4, CD8 counts and reduction in monocytes IL-1 β , IL-6, CD14 and sICAM-1.	84
Rheumatoid arthritis	cM-7412	CD4	The degree of clinical improvement correlated with the percentage of antibody coated CD4 lymphocytes in synovial fluid.	85
Rheumatoid arthritis	CAMPATH-1H (intravenous)	CAMPATH-1	Multicenter, open study of 40 adult patients with refractory rheumatoid arthritis. 85% responded clinically and the response lasted for the mean duration of 2 weeks.	86
Rheumatoid arthritis	CAMPATH-1H (subcutaneous)	CAMPATH-1	Multicenter open study of 30 adult patients with refractory rheumatoid arthritis. 56% responded clinically and the response lasted for the median duration of 32 days.	87
Malignant tumors	M22	Fc receptor of IgG	Destruction of tumor cells under physiologic conditions. No reported clinical trials.	88
Tumor therapy (diagnostic)	h MN-14	Carcinoembryonic antigen (CEA)	A pilot clinical trial in patients with advanced CEA producing tumors showed excellent tumor targeting, biodistribution and pharmacokinetics of h MN-14 similar to the murine version.	89
Breast cancer	hu-Bre-3 hu-Mc 3	Breast epithelial cell	Both humanized mAbs showed reactivity and binding affinity similar to the original murine forms. No reported clinical trials.	90
Breast cancer	CHL6	Breast epithelial cell	Radioiodine labeled CHL6 produced tumor reduction in 50% of chemotherapy-refractory patients with metastatic breast cancer.	91
Colon carcinoma	C242	Colon carcinoma cells	An antibody-superantigen model to recruit tumor infiltrating lymphocytes inducing programmed cell death of tumor cells.	92
Multiple myeloma	AT 13/5	CD38	Lysis of CD38 positive myeloma cells. No reported clinical trials.	93
Myeloid leukemia	Hu M195	CD33	Pretreatment with Hu M195 reduces large number of leukemia cells and better engraftment of bone marrow transplantation.	94
Non-Hodgkins lymphoma	LL2	CD22	Antitumor effect of LL2 detected in 6 out of 21 chemotherapy-resistant patients.	95
Septic shock	CDP571	TNF α	A multicenter, randomized placebo-controlled trial. Rapid decline of TNF- α observed. Mortality rate similar in both placebo and treatment groups.	96
HIV	CD4-IgG & anti-CD3-IgG	CD4, CD3	Bispecific antibody response-targeting and lysis of HIV infected cells.	97
Thromboembolic disorders	YM207	Platelet glycoprotein	The inhibition of platelet aggregation correlated with percentage of platelet glycoprotein receptors blocked.	98
Allergic diseases	Sch 55700	IL-5	Inhibition of eosinophilia in allergic animal models.	99
Asthma	rhu MAb-E25	IgE	A multicenter, randomized, double-blind placebo controlled study in 10 allergic asthmatics. Fall in serum-free IgE and improvement in both methacholine PC ₂₀ and allergen PC ₁₅ demonstrated. One patient was withdrawn due to a generalized reaction after the first dose of antibody.	100
			In a separate study of 19 allergic asthmatic patients, rhu MAb-E25 treatment resulted in reduction of both early- and late-phase responses following allergen inhalation.	101

coming the tumor cell heterogeneity that presents a problem for other monoclonal antibody-mediated approaches.⁷³ The genetic fusion of plant and bacterial toxins with the antibody molecule, the immunotoxins, have been shown to be tumoricidal against

human cancer cells both in vitro⁷⁴ and in vivo⁷⁵ in mouse models. Certain drugs such as Calicheamicin, which has tumoricidal activity, can be linked to antibody and then can be targeted specifically for tumor regression in vivo and for cell killing in vitro.⁷⁶

Humanized Bispecific Antibodies

Two antibodies with different specificity can be linked together to form a product termed bispecific antibody. An antibody of this kind reactivity has been developed with specificity for cytotoxic lymphocytes and tumor cells

overexpressing the HER2 protooncogene.^{77,78} Anti-HER2 consists of one antibody fragment target for breast and ovarian tumor and the other antibody fragment is directed against the CD3 molecule on T cells. Subsequently an improved anti-CD3 variant V9 was identified which binds to T cells with much greater affinity than the original variant, V1.⁷⁹ When interleukin-2 or anti-CD3 activated leukocytes are added to the bispecific antibody conjugate prior to the injection, the reduction of tumor in nude mice is more effective than the activated cells alone.⁸⁰ The bispecific murine antibody 1A10 is targeted against the human transferrin receptor and the human tumor-associated antigen, gp40. This antibody binds to these two antigens on the same tumor cell. Bispecific antibody 1A10 may be useful in malignant disorders.⁸¹ The recombinant methods to produce bispecific antibody fragments have also been developed.^{82,83}

There has been a plethora of recent articles describing the use of antibody. Table 4 outlines many of these studies together with brief comments about their performance.

SUMMARY

The use of antibodies in therapy has progressed as a result of continued development of cellular and molecular biology methods. This is evident from the evolution of multiple generations of antibody drugs, ie, polyclonal antibodies, monoclonal rodent antibodies, and now chimeric or engineered humanized antibodies. Available data suggest that the latter two antibody constructs are less immunogenic and less toxic in humans when compared with rodent mAbs. Their long-term safety can only be ascertained in prospective clinical trials. The therapeutic potential of these "magic bullets" has not been fully explored. It is uncertain, however, if even a humanized antibody can remain invisible to the immune system, since anti-idiotypic and anti-allotypic antibodies can develop. While humanized antibodies present a novel approach for the treatment of a

broad range of diseases including allergic disease and most recently human immunodeficiency viral infection,¹⁰² data obtained in future studies as to their efficacy and safety will determine their ultimate fate as therapeutic agents.

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CME Examination

No 008-008

Questions 1-24, Vaswani SK and RG Hamilton 1998;81:105-119

CME Test Questions

1. The limiting factors of polyclonal antibody treatment are
 - a. Risk of infection from contamination
 - b. Serum sickness
 - c. Anaphylaxis
 - d. Multiple antigen specificities
 - e. All of the above
2. Which of the following is *not* a true statement about monoclonal antibodies (mAbs):
 - a. mAbs can be produced by hybridoma technology
 - b. Lower specificity and affinity than polyclonal antibodies
 - c. With the mAbs, the amount of protein injected is significantly less than with the polyclonal antibody treatment
 - d. They can be easily standardized
 - e. They have proved to be very valuable in laboratory diagnostic methods and therapeutic measures
3. Which of the following are the disadvantages of murine mAbs:
 - a. Elicitation of human anti-mouse immunoglobulin antibody (HAMA) when administered in human body
 - b. Accelerated clearance from the blood and attenuation of therapeutic effect due to neutralization by HAMA
 - c. Risk of hypersensitivity reaction
 - d. Not as effective as human monoclonal antibodies at fulfilling human immune effector functions
 - e. All of the above
4. An antibody molecule consists of:
 - a. Two identical heavy chains
 - b. Two identical light chains
 - c. A variable region
 - d. A constant region

- e. All of the above
5. Humanization of murine mAbs is accomplished by:
- Fusion of cells secreting murine and human antibodies
 - Injection of murine mAb into a human
 - Injection of human antibody into a mouse
 - Substitution of part of the murine antibody with part of a human antibody through a genetic manipulation
 - Transformation of murine mAb into a complete human mAb
6. Which of the following is *not* a true statement about Chimeric or First Generation humanized antibodies:
- They are produced by transplantation of variable region genes of a rodent antibody onto the constant region genes of a human antibody
 - Pure rodent mAbs are less sensitizing than the chimeric antibodies
 - They retain the effector functions of a human antibody
 - They retain the antigen binding capacity of the original rodent mAb
 - Different human antibody isotypes can be selected to produce a chimeric antibody depending on the need for specific effector functions
7. Complementarity Determining Regions (CDRs) are:
- The antigen binding sites
 - Located on variable region of both heavy and light chains
 - Used in the generation of Hyperchimeric or Second generation humanized antibodies
 - Much less immunogenic than the entire variable domain
 - All of the above
8. Complementarity Determining Region (CDR) grafting is:
- Similar to the production of chimeric or first generation humanized antibodies
 - Transplantation of human antibody CDRs onto the rodent mAb
 - Grafting of three CDRs of both heavy and light chains from a rodent mAb into the framework of a human antibody
 - Production of humanized antibodies that are more immunogenic than the first generation chimeric antibodies
 - Production of humanized antibodies that are non-immunogenic
9. Which of the following isotypes used in humanized antibody production is/are most effective in mediating antibody-dependent cellular toxicity (ADCC) and antibody-dependent complement mediated cytotoxicity (ADCMC):
- IgG1
 - IgG2
 - IgG3
 - IgG4
 - both IgG1 and IgG3
10. The isotype least effective in ADCMC and ineffective for ADCC is:
- IgG1
 - IgG2
 - IgG3
 - IgG2 and IgG3
 - IgG1 and IgG3
11. An ideal humanized anti-IgE antibody should be able to:
- Reduce the level of total serum IgE
 - Block the binding of IgE to the mast cells and basophils
 - Reduce the allergy symptoms
 - Reduce the allergic inflammation in asthma
 - All of the above
12. Anti-IgE antibody that binds the FcεRI-bound IgE is:
- an ideal antibody to reduce the total serum IgE level
 - an excellent antibody to reduce the allergic inflammation without potential of any side effects
 - Not an ideal antibody drug since cross-linking of bound IgE on mast cells and basophils results in degranulation and release of mediators of inflammation.
 - useful in reducing the severity of asthma
 - none of the above
13. Humanized antibody with potential for in allergic diseases, should be aimed at following targets except:
- IgE
 - Mast cells
 - Basophils
 - IgG
 - Eosinophils
14. A humanized antibody for potential use in the treatment of allergic inflammation will not be of any use if targeted against:
- Th2 type 'T' lymphocytes
 - Th1 type 'T' lymphocytes
 - VCAM
 - Leukotriene B4
 - Leukotriene C4
15. Anti-VLA4 humanized antibody may be useful in treating allergic inflammation by:
- Preventing the binding of eosinophils to VCAM-1
 - Reducing the eosinophilia
 - Preventing the binding of mast cells to VCAM-1
 - Reducing the levels of IgE
 - Preventing the binding of IgE to the basophil
16. Anti-IL5 mAb (TRFK-5) in mice decreased the number/amount of:
- Eosinophils
 - Basophils
 - Mast cells
 - IgE
 - IgG
17. Which of the following is *not* a true statement about humanized anti-platelet antibody (c7E3 Fab):
- Reduction of number of myocardial infarctions
 - Reduction of the need for emergency coronary revascularization
 - Decreased rates of major bleeding and blood transfusion
 - Reduction of ischemic complications from coronary angioplasty
 - Reduction of ischemic complications from atherectomy
18. CAMPATH-1H is a humanized antibody against:
- Red blood cells
 - Leukocytes
 - Platelets

- d. Mast cells
e. IgE
19. Which of the following is *not* a true statement about the antibody fragments:
- Greater penetration into tumor tissue than the intact antibody molecule
 - More rapid clearance than intact antibody from non-tumor tissue
 - Economical production
 - Non-homogenous binding to tumor tissue
 - Can possess high affinity
20. Humanized Bispecific antibody:
- Is linking of two antibodies with different specificities
 - Is linking of two antibodies with the same specificity
 - Is one antibody molecule with specificity for two different antigens
 - Is specific conjugation of murine and human antibodies
 - Is an antibody used to kill two different types of specific lymphocytes
21. All of the following antibodies have been studied in the clinical trials for Rheumatoid Arthritis *except*:
- CAMPATH-1H subcutaneously
 - Anti-CD4 antibody
 - TNF- α blocking antibody
 - CAMPATH-1H intravenously
 - Anti-Rheumatoid Factor
22. Which of the following is a *true* statement about the antibody targeted superantigen therapy:
- Consists of a fusion of superantigen with the antibody
 - Causes recruitment of tumor infiltrating lymphocytes
 - Induces release of cytokines by the lymphocytes
 - Fosters programmed cell death of the malignant cells
 - All of the above
23. In animal model, humanized anti-IL5 antibody (Sch 55700) inhibits the number/amount of:
- Eosinophils
 - Mast cells
 - B cells
 - T cells
 - IgE
24. The scope of humanized antibody therapy is limited to:
- Allergic diseases
 - Malignancy
 - Infectious diseases (HIV, Sepsis)
 - Thromboembolic disorders
 - All of the above. The therapeutic potential of humanized antibodies appears to be unlimited.

Answers to CME examination—Annals of Allergy, Asthma, & Immunology, June 1998 (Identification No 008-006) Stenton GR et al: Role of intestinal mast cells in modulating gastrointestinal pathophysiology. Ann Allergy Asthma Immunol 1998;80:1-15.

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|------|-------|-------|-------|
| 1. e | 6. e | 11. a | 16. c |
| 2. d | 7. d | 12. b | 17. d |
| 3. d | 8. b | 13. d | 18. c |
| 4. c | 9. c | 14. c | 19. c |
| 5. c | 10. d | 15. d | 20. a |

Answers to CME examination—Annals of Allergy, Asthma, & Immunology, July 1998 (Identification No 008-007) Busse W: The role and contribution of leukotrienes in asthma. Ann Allergy Asthma Immunol 1998;81:17-29.

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|------|-------|-------|-------|
| 1. c | 6. e | 11. d | 16. d |
| 2. c | 7. a | 12. a | 17. e |
| 3. b | 8. b | 13. c | 18. b |
| 4. c | 9. a | 14. d | 19. b |
| 5. a | 10. d | 15. b | 20. c |

CASE 4-30583A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

Art Unit: 1644

AMLOT ET AL.

Examiner: Gerald Ewoldt

APPLICATION NO: 09/770,002

FILED: JANUARY 25, 2001

FOR: USE OF CD25 BINDING MOLECULES IN THE TREATMENT OF
RHEUMATOID ARTHRITIS OR SKIN DISEASESCommissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450PETITION FOR EXTENSION OF TIME

Sir:

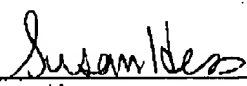
The Office Action of April 28, 2003 has a shortened statutory time set to expire on July 28, 2003. A three-month extension is hereby requested pursuant to 37 CFR §1.136(a).

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of \$930 for payment of the extension fee. An additional copy of this paper is here enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.17 which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

Respectfully submitted,

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Date: October 28, 2003



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Case No. 4-30583A
Application No. 09/770,002
Mailing Date: October 28, 2003
Due Date: October 28, 2003
Express Mail No.: EV335543080US

The Patent & Trademark Office acknowledges, and has stamped hereon the date of receipt of the items checked below:

☐ Amendment/Response/Letter - Fee \$ 1

☐ Appln. Filing Papers - Fee \$

☐ PCT National Stage

☐ Provisional Application

☐ RCE ☐ DIV ☐ CONT ☐ CIP

☐ Specification Pg's

☐ Executed/Unexecuted Decl. - Fee \$

☐ Missing Parts/Missing Req.

☐ Preliminary Amendment Pg's

☐ Claim of Priority ☐ Certified Copy(s)

☒ Amendment After Final (6 Pages)

☐ Notice of Appeal - Fee \$

☐ Appeal Brief - Fee \$

☐ Issue Fee Payment \$

☐ Assignment Rec. Req. - Fee \$

☐ Formal Drawings Pg's

☐ IDS Pg's - Fee \$

☐ PTO-1449 Form Pg's

☒ Pet. for Ext. of Time - Fee \$ 930.00

☐ Application Data Sheet

☐ Seq. Listings Pg's/Seq. Disk

☒ one Reference

☐ SH

Initials SH T02 Rec'd PCT/PTO 28 OCT 2003

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